New and Notable

Clamping Down on Tumor Proliferation

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Mechanical compression initiates an unknown proliferation constraint in the center of tumor spheroids. In the future, clinicians could consider activating the stroma to regulate tumor progression.

The origin and growth of tumors is increasingly recognized to depend on the physical properties of cells and tissues, and not just on their genetics and biochemistry. Most recent experimental studies have focused on the altered viscoelasticity of cancer cells and their extracellular matrices, but mechanical stresses that arise as malignant cells grow within confined volumes are equally important, even if they are harder to study in vitro. Montel et al. (1), in 2011, reported a new technique to apply a compressive mechanical stress to a cell spheroid. The technique they use is similar to traditional high osmolarity shocks, which lead to volume reduction through water loss, but with one important difference: it does not utilize soluble salts, which can be transported across the cell membrane, significantly impacting the biochemistry and signaling of the single cell. Instead, they add to the media Dextran, a high-molecular-weight polysaccharide that is not taken up or digested by the cells. This means that only the outer cells of the spheroid are affected osmotically, and they act like a compressing shell on the inner cells. This technique is therefore a stress clamp, using the outer cells as the vise (Fig. 1).

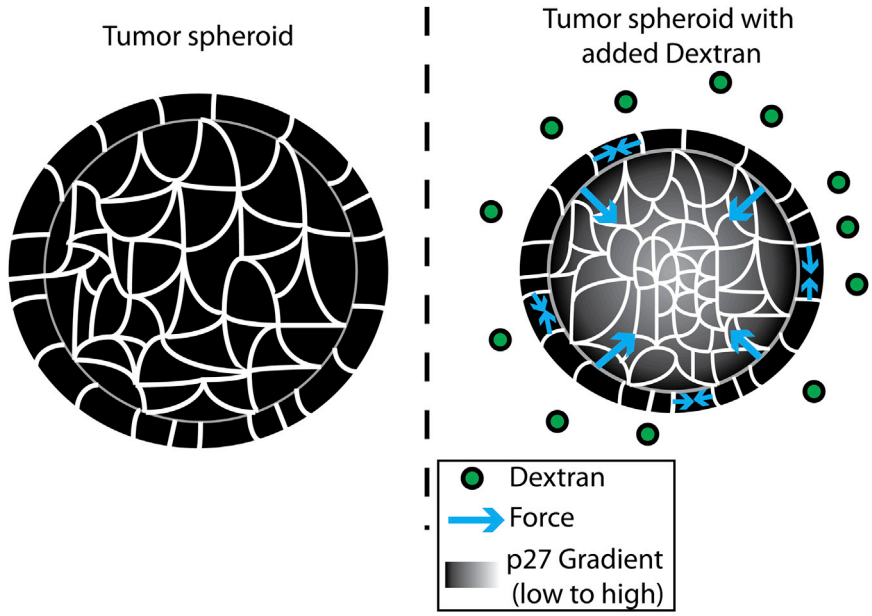

FIGURE 1 Schematic of the stress-clamping technique. Large dextran molecules added to the medium of a tumor spheroid cause compression of the tumor spheroid, causing an upregulation of p27 in the center of the spheroid. To see this figure in color, go online.

Externally applied or internally generated stress has long been thought to play an important role in the regulation of tumor growth (2), and tumor spheroids are an effective method of testing this in vitro. In this issue of the *Biophysical Journal*, Delarue et al. (3) use their stress-clamping technique to test the effect of mechanical compression on cell proliferation in tumor spheroids. When the compressive stress is on the same level as the Young's modulus of the spheroid, they observe an immediate and recoverable reduction in cell volume in the middle of the tumor spheroid. This is followed after several hours by an upregulation of $p27^{Kip1}$ (p27), a protein that blocks the cell cycle at the late G1 checkpoint, in those same centrally located cells. Consequently, on the timescale of days, there is an observable reduction in cell proliferation in the center of the spheroid. Furthermore, the upregulation of p27 is caused directly by the mechanical compression and/or volume loss in the central cells. Moreover, when p27 is silenced, the cell volume still decreases when the spheroid is compressed, but there is no observable reduction in proliferation. This suggests a causal link between mechanical compression and p27-mediated reduction of proliferation in the center of the spheroid.

There are two potentially important results in this article, all of which open new avenues for exploration.

First, the mechanical clamping technique of Delarue et al. (3) promises a means to apply mechanical compression without either adding cell-permeant solutes to the media or directly contacting the cells with an external loading device. However, the detailed mechanism of compression requires elucidation, in so far as they do not observe significant volume reduction at the level of single cells, yet there is a clear but small osmotic impact on the outer cells of the spheroid. This discrepancy is likely the result of a multiplicative effect of very small

Submitted August 21, 2014, and accepted for publication September 3, 2014.

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Editor: Ewa Paluch.

0006-3495/14/10/1775/2 $2.00

http://dx.doi.org/10.1016/j.bpj.2014.09.004

volume changes across outer cells or loss of fluid from the intercellular space; future experiments with greater resolution on live cells will help unveil the basis for the compression.

Second, the mechanism by which p27 is activated is still unknown. The authors postulate that it could be through the FoxO family pathway, which has a direct inhibitory link to p27 (4). Interestingly, the FoxO pathway is regulated in part by calcium activation, and calcium channels can respond to mechanical forces (5) including compression (6). It will be interesting to see if there is any link between p27 and other known mechanosensitive pathways, such as the Hippo pathway and Yap/Taz (7).

Ultimately, one of the most enticing future possibilities offered by this study is that p27, and any other mechanism through which proliferation is controlled, is sensitive to compressive stress. A connection between compression and proliferation is not unique to cancer, and Delarue et al. (3) show that it applies to both cancer and normal cells, although it is intriguing that the antiproliferative effect of compression appears to be stronger for Schwann cells than for the four cancer cell types studied. It has been known for some time that p27 is sensitive to stretch (8), but in cancer and development, compression is at least as important a microenvironmental control. New studies highlight the importance of compressive stress to mechanosensitivity (9) in general, and specifically in cancer (10) and stem cells (11). Important for clinicians is the possibility that this link between compression and proliferation may highlight another opportunity for control of tumor growth, which is entirely physical in origin.

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